1: IUBMB Life. 2002 Mar;53(3):161-6.

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LAST motifs and SMART domains in gene 32 protein: an unfolding story of autoregulation?

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Bacteriophage T4 gene 32 protein is a classical single strand-specific DNA binding protein. It is a single polypeptide chain of 301 amino acid residues that consists of three structural domains, each of which has a binding function. The N-terminal domain is involved in homotypic protein-protein interaction (the basis of binding cooperativity), the core domain binds single strands directly, and the C-terminal domain has a role in heterotypic protein-protein association. The three domains have traditionally been thought to be independent of each other. However, the observation of a striking repetition of a basic, polar sequence (the "LAST" Motif), seen in both the N-terminal and core domains, suggests a linkage between these domains. Moreover, the C-domain and adjoining portion (flap) of the core are highly acidic, and are potential mimics of single-stranded DNA. With these observations, I construct a model in which this flap is associated with the ssDNA binding site in the absence of DNA, and upon cooperative protein binding to DNA, the flap now associates with the N-terminal domain of the adjacent DNA-bound protein. The flap thus acts as a gate, which might slow the binding of the protein to DNA. This could lead to the regulation of the protein's various interactions with other proteins, as well as affect its ability to lower DNA melting temperature.

PMID: 12102172 [PubMed - indexed for MEDLINE]

1: Mol Microbiol. 2005 Mar;55(5):1502-14.

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Protein-DNA interactions in the T4 dNTP synthetase complex dependent on gene 32 single-stranded DNA-binding protein.

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Our laboratory has reported data suggesting a role for T4 phage gene 32 single-stranded DNA-binding protein in organizing a complex of deoxyribonucleotide-synthesizing enzymes at the replication fork. In this article we examined the effects of gene 32 ablation on the association of these enzymes with DNA-protein complexes. These experiments showed several deoxyribonucleotide-synthesizing enzymes to be present in DNA-protein complexes, with some of these associations being dependent on gene 32 protein. To further understand the role of gp32, we created amber mutations at codons 24 and 204 of gene 32, which encodes a 301-residue protein. We used the newly created mutants along with several experimental approaches--DNA-cellulose chromatography, immunoprecipitation, optical biosensor analysis and glutathione-S-transferase pulldowns--to identify relevant protein-protein and protein-DNA interactions. These experiments identified several proteins whose interactions with DNA depend on the presence of intact gp32, notably thymidylate synthase, dihydrofolate (DHF) reductase, ribonucleotide reductase (RNR) and Escherichia coli nucleoside diphosphate (NDP) kinase, and they also demonstrated direct associations between gp32 and RNR and NDP kinase, but not dCMP hydroxymethylase, deoxyribonucleoside monophosphate kinase, or DHF reductase. Taken together, the results support the hypothesis that the gene 32 protein helps to recruit enzymes of deoxyribonucleoside triphosphates synthesis to DNA replication sites.

PMID: 15720556 [PubMed - in process]

1: J Biol Chem. 2000 Oct 6;275(40):31496-504.

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Mutations in the N-terminal cooperativity domain of gene 32 protein alter properties of the T4 DNA replication and recombination systems.

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The gene 32 protein (gp32) of bacteriophage T4 is the essential single-stranded DNA (ssDNA)-binding protein required for phage DNA replication and recombination. gp32 binds ssDNA with high affinity and cooperativity, forming contiguous clusters that optimally configure the ssDNA for recognition by DNA polymerase or recombination enzymes. The precise roles of gp32 affinity and cooperativity in promoting replication and recombination have yet to be defined, however. Previous work established that the N-terminal "B-domain" of gp32 is essential for cooperativity and that point mutations at Arg (4) and Lys(3) positions have varying and dramatic effects on gp32-ssDNA interactions. Therefore, we examined the effects of six different gp32 B-domain mutants on T4 in vitro systems for DNA synthesis and homologous pairing. We find that the B-domain is essential for gp32's stimulation of these reactions. The stimulatory efficacy of gp32 B-domain mutants generally correlates with the hierarchy of relative ssDNA binding affinities, i.e. wild-type gp32 approximately R4K > K3A approximately R4Q > R4T > R4G gp32-B. However, the functional defect of a particular mutant is often greater than can be explained simply by its ability to saturate the ssDNA at equilibrium, suggesting additional defects in the proper assembly and activity of DNA polymerase and recombinase complexes on ssDNA, which may derive from a decreased lifetime of gp32-ssDNA clusters.

PMID: 10906124 [PubMed - indexed for MEDLINE]